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Effects of plant tannin supplementation on animal responses and in vivo ruminal bacterial populations associated with bloat in heifers grazing wheat forage[1]

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ABSTRACT

Two experiments were conducted to 1) enumerate the effect of tannin supplementation on ADG, bloat frequency, in vitro gas, and biofilm and foam production, and 2) quantify the influence of tannin supplementation on ruminal bacterial populations of heifers grazing wheat forage. Twenty-six heifers (286 ± 26 kg) were randomly allocated to 1 of 3 treatments that included a control (nontannin group) and 2 types of tannins (mimosa and chestnut tannins). Plant tannins (1.5% of DMI) were supplemented once daily mixed with a textured feed (500 g/animal). Heifers were visually monitored daily for bloat score (0 = no bloat, 3 = severe bloat). In Exp. 1, supplementation of tannins reduced the rate of frothy foam and biofilm production with chestnut being more efficacious than mimosa tannins. There was no treatment × time interaction ($P > 0.1$) for ADG and bloat measurement. Chestnut tannin supplementation increased ADG ($P < 0.05$) with time ($P < 0.01$). Mean bloat score and bloat day were greater ($P < 0.01$) for the control diet than for tannin-supplemented treatment groups. In Exp. 2, Fibrobacter succinogenes, Streptococcus bovis, and Prevotella ruminicola strains were relatively stable with time (d 0, 10, and 25) in the rumen of animals not receiving tannin supplementation. However, with supplementation of chestnut and mimosa tannins, populations of P. ruminicola and strains of both F. succinogenes and Ruminococcus flavefaciens were increased, respectively. Results show that daily supplementing mimosa and chestnut tannins to heifers grazing wheat forage improved beef production and minimized bloat frequency with minimum impact to the microbial populations studied.

Key words: bacterial population, bloat, tannin

INTRODUCTION

It has been found over a 3-yr period that increasing bloat severity of stocker cattle grazing winter wheat forage negatively ($R^2 = 0.45$) affected ADG in a linear fashion {W. E. Pinchak, B. R. Min, D. P. Malinowski [AgriLife Research, Texas A&M System, Vernon, TX], J. W. Sij [AgriLife Research, Texas A&M Sys-

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tem, Vernon, TX], J. D. Fulford, and R. Puchala [E. (Kika) de la Garza American Institute for Goat Research, Langston, OK], unpublished data}. Frothy bloat is caused by high solubility of wheat forage proteins leading to the development of stable foam and polysaccharide biofilm in the rumen that traps rumen gases (Min et al., 2005b). Therefore, altering the rapid rate of ruminal fermentation by decreasing biofilm and gas production in the rumen may be central to wheat pasture bloat mitigation. Condensed tannin-containing forages or tannin supplementation in grazing cattle have been previously shown to decrease bloat potential and increase ADG (Jones and Lyttleton, 1969; Min et al., 2006b). The effect of tannin supplementation are likely caused by a combination of the reduced ruminal gas and foam production by precipitating the stable forage protein foam, antimicrobial action of tannins, and increased ruminal escape protein (Chiquette et al., 1988; Min et al., 2005b). However, there is a need to assess different sources of tannins (condensed vs. hydrolysable tannins) to determine the balance of efficacious dose and potential deleterious effects of the tannin extracts on animal performance.

Ruminal microbial activity probably contributes to frothy bloat in cattle grazing bloat-prone legumes (e.g., alfalfa and white clover) and wheat forage through the formation of low-gas permeable biofilms. Grazing wheat forage and frothy bloat in cattle grazing wheat often results in changes in the relative composition of bacterial populations through time (Min et al., 2006a,b). Min et al. (2005b) found that ruminal gas and biofilm production were inhibited when quebracho condensed tannin extracts were supplemented to in vitro incubations of mixed ruminal fluid. The implication of biofilm production likely being associated with specific rumen microorganisms (Min et al., 2006b) led us to study the effect of 2 contrasting plant tannins on ruminal microbial diversity associated with bloat severity. The primary hypothesis of the in vitro and in vivo research was that different sources of tannin supplementation (mimosa vs. chestnut tannins) would reduce rumen microbial activity, ruminal gas production, and as a result would decrease the incidence of bloat and increase ADG in heifers grazing wheat forage. The objectives of this experiment were to determine changes in 5 rumen bacterial profiles through grazing time associated with frothy bloat and ADG in response to plant tannins.

MATERIALS AND METHODS

Experimental Design

All procedures related to the animals used in the current study were accepted by the Institutional Animal Care and Use Committee at Texas A&M University, and the animals were cared for according to its guidelines. An initial grazing study was conducted to quantify the effect of plant tannin supplementation on biofilm production, in vitro ruminal gas and foam production, animal growth responses, and bloat frequency in heifers grazing winter wheat forage. A second in vivo experiment was designed to quantify the effect of tannin supplementation on ruminal bacterial diversity dynamics in heifers grazing wheat forage.

Experiment 1: Grazing and In Vitro Rumen Fermentation Experiment

In the in vivo experiment, 26 healthy heifers (Angus × Hereford × Brangus; 286 ± 26 kg) were used to quantify biofilm complexes, in vitro gas production, ADG, and ruminal bacterial populations associated with frothy bloat during the peak bloat occurrence period from February 18 to March 19, 2007, on grazing wheat forage (*Triticum aestivum* L. var. "Cutter"). Animals were randomly allocated to 1 of 3 treatments that included a control (texture feed only) and 2 types of tannins (textured feed with added mimosa or chestnut tannin extracts). Animals were received a textured feed (500 g/heifer) with or without plant tannins. Plant tannins (1.5% of estimated DMI) were supplemented once daily (approximately 0800 h) mixed with a textured feed on an as-fed basis. The mixed ration consisted of steam-flaked milo (85 kg), hominy feed (10 kg), and molasses (5 kg/100 kg DM) on an as-fed basis. Cattle were weighted at 10-d intervals without shrink adjustment in the early morning within a 30-min period. From February 18 through March 19, 2007, cattle were visually monitored daily (at 0800 h) and scored for bloat (0 = no bloat, 3 = severe bloat; Paisley and Horn, 1998).

Tannin Chemistry

Approximately 89% of the chestnut tannin (*Castanea sativa* Mill) components are the hydrolysable tannins (Mayer et al., 1967; Viviers et al., 1983), but mimosa tannins (*Acacia mearnsii*; black wattle) contained mainly condensed tannins (70%; Chemtan, Exeter, NH; Zimmer and Cordesse, 1996; Romani et al., 2006). For the in vivo study, both mimosa and chestnut tannins were chosen because the dominant chemical structure (condensed vs. hydrolysable tannins) differed and chestnut tannins have demonstrated more antimicrobial potential than mimosa tannins (Min et al., 2007).

Forage Measurement and Rumen Fluid Sampling

Forage biomass was measured at weekly intervals. Forage allowance was estimated by hand-clipping wheat standing crop from five 1-m^2 quadrats/paddock to ground level. Samples were dried in a forced-air oven at 60°C for 48 h. In addition, 3 hand-clipped forage samples (approximately 500 g, fresh basis) for nutritive value analyses were collected from random locations in each paddock. These samples were subsequently pooled, thoroughly mixed, and analyzed for protein fractions. Subsequent forage samples were stored at −20°C for in vitro ruminal gas production analyses or oven-dried

in a forced-air oven at 60°C for 48 h and ground (Cyclone sample mill, Udy Co., Fort Collins, CO) to pass a 1-mm sieve for NDF and IVDMD analyses. Fresh frozen forage was minced in a blender (model DS-7, Waring Products Co., Winsted, CT) for use in in vitro experiments.

Rumen content for in vitro gas production, biofilm production, and PCR analysis were collected on d 0, 10, and 25 after an initial 4-wk (January 18 to February 18, 2007) wheat grazing adaptation period. Rumen contents were collected 2 h post-tannin supplementation. Rumen fluid was assayed using the method described by Min et al. (2006a). Samples were obtained (about 60 mL) from selected 4 animals among 8 heifers in each treatment using a stomach tube and transported to the laboratory within 30 min of collection. Rumen samples were allocated for in vitro gas and frothy foam production (20 mL), biofilm production (30 mL), and DNA extraction (10 mL). Polysaccharide biofilm production by rumen microorganisms has been considered as one of the primary causative factors to a stable foam formation, which interferes with the normal eructation of gases, contributing to frothy bloat (Bryant et al., 1960; Gutierrez et al., 1963).

The rumen fluid was immediately placed on 50-mL serum vials that were capped and returned to the laboratory for determination of specific activities of foam and gas production. Specific activity of foam and gas production were determined by combining (18 × 150 mm crimp-top tubes) 5 mL rumen fluid (strained with by 4 layers of cheese cloth) from each heifer with 5 mL anaerobic artificial saliva (McDougall, 1948) containing 0.2 g finely ground wheat forage (to pass 2-mm sieves). Forage was collected (hand-pluck) from the same experimental units. These tubes were capped, attached with 60-mL syringe, and incubated at 39°C under CO_2 atmosphere. In vitro gas production was measured as plunger displacement (cm^3) at 0, 1, 2, 3, 4, 5, 6, 8, and 12 h incubation periods (Min et al., 2005c), as well as ruminal foam production (cm^3/h) inside test tubes at the same time periods measured by electrical digital caliper (Control Company, Friendswood, TX). In vitro incubation was under taken in duplicate for each time period. Total in vitro gas produced was corrected to blank incubations (i.e., no ruminal fluid).

Biofilm Measurement

The biofilm complexes (mg DM/mL) in all experiments were measured as described by Gutierrez et al. (1963). Rumen samples were strained with 4 layers of cheesecloth to remove most of the large debris and centrifuged in a microcentrifuge (Sorvall Centrifuge, Kendro Laboratory Products, Langenselbold, Germany) at 16,000 × *g* for 30 min to remove most of the bacteria, protozoa, and small debris. To the supernatant rumen fluid (1 mL) was added 1 mL of absolute ethanol to precipitate a viscid material, and kept at 5°C for 24 h. Precipitate was harvested by centrifugation at 16,000 × *g* for 15 min. The biofilm fraction was dried for 24 h at 60°C and weighed for DM determination.

Experiment 2: Rumen Microbial Analysis, and Bacterial Culture and Growth Medium

Five strains of ruminal bacteria were used for PCR experiments to determine the effect of dietary plant tannins on specific bacterial population dynamics in the rumen of steers grazing wheat forage. The following strains of rumen bacteria were used as reference strains: *Fibrobacter succinogenes* S85, *Ruminococcus flavefaciens* C94, *Streptococcus bovis* strain 26, *Prevotella ruminicola* strain 23, *Eubacterium ruminantium* B1C23. All isolates were inoculated into anaerobic basal medium in Hungate tubes for 24 h at 39°C from their respective long-term storage vials (lyophilization). The isolates were re-inoculated into anaerobic wheat-soluble plant protein medium and incubated for 24 h at 39°C (Min et al., 2006b). Preparation, distribution, and inoculation of a basal growth medium containing wheat-soluble plant protein medium were carried out according to the Min et al. (2006b).

DNA Extraction and Analysis of PCR

Genomic bacterial DNA was isolated from 1 mL of each unknown rumen sample according to the method described in the QIAamp DNA Mini Kit (Qiagen, Valencia, CA). Concentrations of DNA were measured using a NanoDrop Spectrophotometer (ND-1000, Wilmington, DE). The primers designated to detect the target species (Tajima et al., 2001) are listed in Table 1. The PCR amplifications were conducted using species-specific PCR primers. To minimize animal-to-animal variations, the aliquots of DNA samples from 4 animals were mixed after DNA extraction.

The PCR amplifications were performed on the PTC-200 Peltier Thermal Cycler (MJ Research Inc., Waltham, MA) with the following program: 1) denaturation at 95°C for 3 min; 2) 35 subsequent denaturing cycles at 95°C for 30 s; 3) various annealing temperatures (described in Table 1) for 30 s, and extension at 72°C for 1 min (Tajima et al., 2001). Primers [50 pmol of each per reaction mixture; primer 2, and primer 3 (Integrated DNA Technologies Inc., Coralville, IA; Sheffield et al., 1989; Muyzer et al., 1993)] were mixed with Jump Start Red-Taq Ready Mix (Sigma Chemical Company, St. Louis, MO), according to the kit instructions: 250 ng of template DNA from rumen digesta of pooled steers, and 5% (wt/vol) acetamide to eliminate preferential annealing (Reysenbach et al., 1992). The PCR products were separated by electrophoresis on 2% precast agarose E-gel system (Invitrogen, Carlsbad, CA). The 16S rDNA isolated from the rumen of heifers (n = 4) grazing winter wheat forage with or without tannin supplementation was PCR-amplified using species-specific primers (Table 1) and resulting

Table 1. Polymerase chain reaction primers for detection of rumen bacteria

Target bacteria	Primer	AT,[1] °C	PS,[2] bp
Fibrobacter succinogenes 85	Forward: GTATGGGATGAGCTGC Reverse: GCCTGCCCTGAACTATC	62	445
Ruminococcus flavefaciens C94	Forward: GGACGATAATGACGGTACTT Reverse: GCAATCTGAACTGGGACAAT	62	835
Streptococcus bovis strain 26	Forward: CTAATACCGCATAACAGCAT Reverse: AGAAACTTCCTATCTCTAGG	57	869
Prevotella ruminicola S-23	Forward: GGTTATCTTGAGTGAGTT Reverse: CTGATGGCAACTAAAGAA	53	485
Eubacterium ruminantium B1C23	Forward: GCTTCTGAAGAATCATTTGAAG Reverse: TCGTGCCTCAGTGTCAGTGT	57	671

[1]AT = annealing temperature.
[2]PS = product size.

products were separated on 48-well 2% agarose e-gels (Figure 1). The resulting primer set (Table 1) produced PCR products of the expected size with test strains (Figures 2 and 3).

Chemical Analysis

Total CP, soluble protein-N, insoluble protein-N, and nonprotein-N from fresh forage samples were determined by the Kjeldahl digestion procedure (AOAC, 1990). Fresh forage samples were prepared as described by Bartley et al. (1975) and Min et al. (2005a). One gram of the chopped (about 0.5 cm) plant material from each sample was analyzed for Kjeldahl N (total N). Fresh wheat forage was fractionated into soluble protein-N and soluble NPN by blending 5 g of sample with 100 mL of distilled water, and chopped (approximately 1,000 × *g*) for two 15-s intervals with a blender (Warning Products Co.). Homogenate was vacuum filtered through Whatman number 4 filter paper. The residue was transferred to Kjeldahl flasks to determine insoluble protein. Eighty milliliters of the measured filtrate was acidified by the addition of 10 mL of 150 g/L trichloroacetic acid to precipitate soluble N (Waghorn and Jones, 1989), and refrigerated (4°C) overnight. The mixture was vacuum filtered through Whatman number 2 filter paper and the filtrate transferred to Kjeldahl flasks to determine nonprotein N. Soluble protein-N was calculated as soluble protein-N = total N – (NPN + insoluble-N). Forage samples and ruminal contents

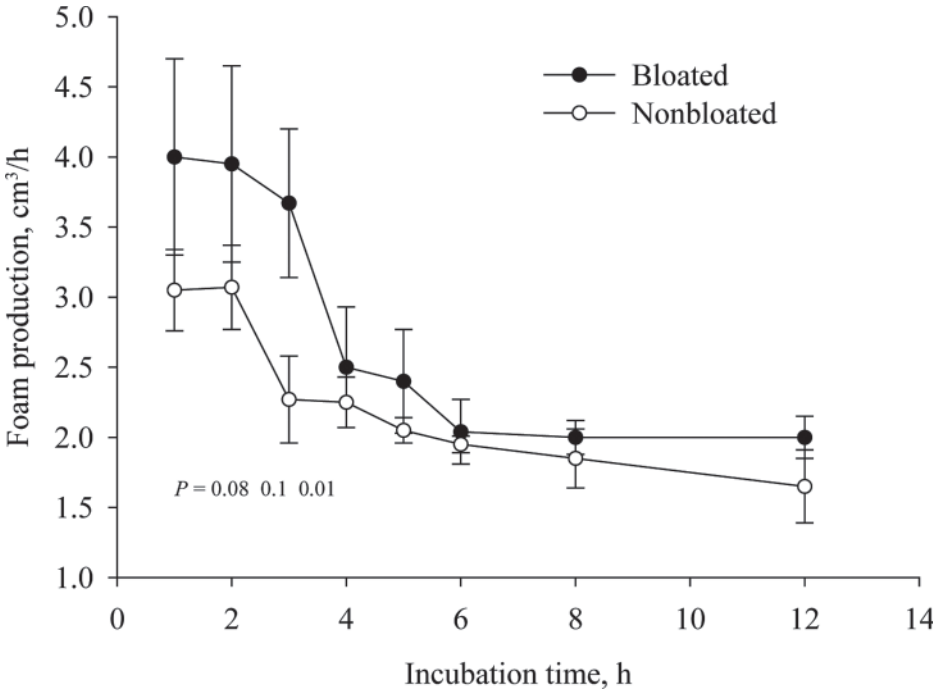

Figure 1. Effects of bloat severity on the in vitro foam production (n = 4) incubated at 37°C with or without bloated rumen fluid collected from heifers grazing wheat forage. Specific activity of foam production was determined by combining, in 18 × 150 mm crimp-top tubes, 5 mL of rumen fluid from each heifer (bloated vs. nonbloated) with 5 mL of anaerobic artificial saliva containing 0.2 g of finely ground wheat forage, which was collected from same experimental units.

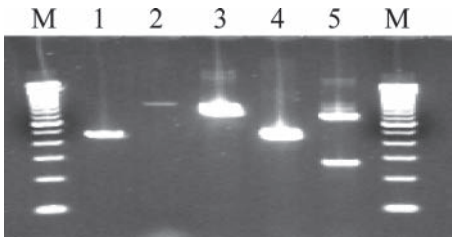

Figure 2. Amplification of control rumen bacterial DNA (strains are listed in Materials and Methods) with the primer set detailed in Table 1. Lanes: 1, *Fibrobacter succinogenes*; 2, *Ruminococcus flavefaciens*; 3, *Streptococcus bovis*; 4, *Prevotella ruminicola*; 5, *Eubacterium ruminantium*. Lane M, DNA size marker.

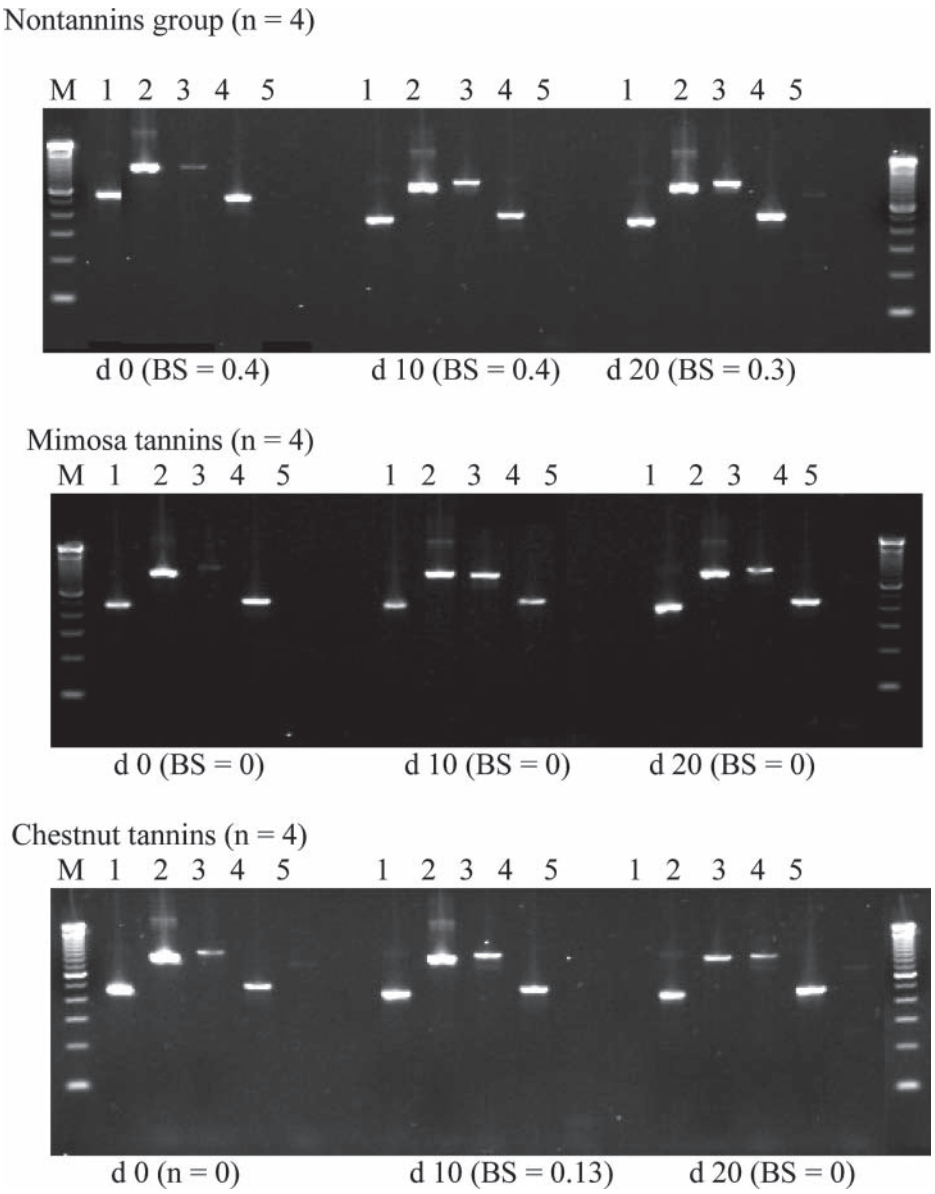

Figure 3. Qualitative PCR detection of 5 bacterial strains in the rumen of heifers grazing winter wheat forage on d 0, 10, and 25 after a 4-wk adaptation period with and without tannin supplementation. BS = mean bloat score. Lanes: 1, *Fibrobacter succinogenes*; 2, *Ruminococcus flavefaciens*; 3, *Streptococcus bovis*; 4, *Prevotella ruminicola*; 5, *Eubacterium ruminantium*. Lane M, DNA size marker.

were oven-dried at 60°C to a constant weight. The NDF, ADF, and IVDMD of dried forage samples were determined using the filter bag technique (Ankom Technology Corp., Macedon, NY).

Statistical Analysis

Data were analyzed as a completely randomized design to test 3 treatments (Tables 2, 3, and 4) using the MIXED procedure of SAS Institute Inc. (SAS, 1990). Data are presented as mean values, together with the SEM. The variables in experiment included forage chemical composition, in vitro ruminal gas and foam production, source of rumen fluid, ADG, bloat frequency, and ruminal biofilm production. Frothy foam production (cm^3/h) and in vitro gas production (bloated vs. nonbloated steers) in heifers grazing winter wheat forage in response bloat score were determined using the MIXED procedure of SAS Institute Inc. (SAS, 1990; Table 4).

The 16S rDNA isolated from the rumen of heifers (n = 4) grazing winter wheat forage with or without tannin supplementation was used. However, to minimize animal-to-animal variations, the aliquots of DNA samples from 4 animals were mixed after DNA extraction; hence, only the phenotypic visual analysis per each strain on each time point is reported. The model included treatments (mimosa vs. chestnut tannins), time, and treatment × time interactions.

An in vitro gas production rate was measured repeatedly and calculated using the exponential equation of Ørskov and McDonald (1979): $Y = a + b(1 - e^{-ct})$, where Y was defined as gas production in time t; a, b, and c being constants of the exponential equation, where a = the gas production at time 0; b = the proportion of gas production during time (t); and c = the rate of gas production of the b fraction. The constants b and c for each treatment were calculated with the method described by Min et al. (2005b) using the nonlinear regression (NLIN) procedure from SAS Institute (SAS, 1990). Cumulative in vitro gas production in each time point was analyzed using the MIXED procedure of SAS Institute (SAS, 1990). Results are reported as least squares means, and significance was declared at $P < 0.05$. There were no treatment × time interactions ($P > 0.10$); hence, only the main effects are reported in the Results and Discussion section. Unequal numbers of animals were used due to shortage of animals during the experimental periods.

RESULTS AND DISCUSSION

The main findings in this study indicated that plant tannin supplementation reduced bloat severity and increased ADG principally through reducing the rate of rumen fermentation as well as modification of microbial population dynamics in the rumen of cattle. Different types of tannins have different animal responses on ADG and rumen bacterial population. This suggests that plant tannin supplementation is effective for

Table 2. Effects of plant tannins on the ADG and bloat frequency in heifers grazing wheat forage during the vegetative stage of growth (February 18 to March 19)

	Dietary treatment				
Item[1]	Control	Mimosa	Chestnut	SEM	*P*-value
Number of heifers	10	8	8		
BW (kg)					
Initial BW	285.5	283.1	289.6	25.7	0.83
Final BW	399.5[b]	408.7[b]	434.8[a]	37.4	0.10
Total gain	69.3	73.3	83.6	6.44	0.18
ADG, 30 d	1.97[b]	2.09[ab]	2.38[a]	0.251	0.05
Time					0.001
Treatment					0.05
Time × treatment					0.97
Bloat measurement					
Mean bloat day	7.4[a]	1.7[b]	1.4[c]	1.36	0.01
Mean bloat score	0.31[a]	0.05[b]	0.05[b]	0.008	0.01
Time					0.50
Treatment					0.01
Time × treatment					0.70

[a–c]Within a column, means without a common superscript letter differ, $P < 0.05$.

[1]Least squares means for the ADG and bloat frequency in heifers grazing wheat forage with or without plant tannin supplementation.

mitigating frothy bloat and improving animal performance on heifers grazing winter wheat forage.

The ADG and bloat dynamics in heifers grazing winter wheat forage in response to tannin supplementation are shown in Table 2. Initial BW of heifers was similar among tannin treatments, but final BW ($P = 0.10$) tended to be greater for chestnut tannins than for other treatments. There was no treatment × time interaction ($P > 0.1$) for ADG and bloat measurement. Chestnut tannin supplementation increased ADG ($P < 0.05$) with time ($P < 0.01$). Mean bloat score and bloat day were greater ($P < 0.01$) for the control diet than for tannin-supplemented treatment groups. Heifers fed 1.5% tannin supplementation experienced 83 to 81% fewer days of bloat, and had 6 and 17% greater ADG for mimosa and chestnut extract tannins, respectively, than animals receiving the control diet. The highly soluble plant protein content and onset of specific rumen bacterial activity have been identified as precursors to bloat on wheat pasture (Bartley et al., 1975; Min et al., 2006b). The presence of plant tannins in the diet can reduce proteolysis, foam, and biofilm production in the rumen due to precipitation of soluble protein:tannin complexes decreases RDP and increases undegradable protein (Min et al., 2003, 2006a) and through antimicrobial activity of tannins toward gram-positive bacteria (Min et al., 2008). The combination of increasing rumen escape protein, reducing ruminal gas, and decreasing foam production contributed to the 6 to 17% increase in ADG observed with mimosa and chestnut tannin supplementation of heifers in this study.

To further understand the effect of mimosa and chestnut tannin supplementation on in vitro ruminal gas production, biofilm and foam production were examined. In vitro rate of gas and potential foam production were similar among control and tannin treatments (Table 3); potential gas production ($P = 0.11$) tended to be lower and rate of foam production ($P < 0.05$) was lower for chestnut tannins than for the control group.

Table 3. Effects of source of ruminal fluid from steers fed plant tannins on in vitro ruminal gas production and biofilm production when incubated with ground wheat forage substrate and mixed rumen microorganisms obtained from heifers grazing wheat forage during the vegetative stage of growth

	Dietary treatment				
Item[1]	Control	Mimosa	Chestnut	SEM	*P*-value
Ruminal gas production					
Rate, c (cm^3/h)	2.60	2.26	2.11	0.34	0.20
Potential, a+b (cm^3/12 h)	35.1	35.1	34.7	0.16	0.11
Frothy foam production					
Rate, c (cm^3/h)	0.96[a]	0.79[ab]	0.76[b]	0.091	0.05
Potential, a+b (cm^3/12 h)	19.1	18.1	18.1	1.75	0.59
Biofilm production (mg of DM/mL)	1.77	1.26	1.54	0.379	0.16

[1]Least squares means (n = 4) for in vitro rumen fermentation with plant tannin treatment.

Table 4. Effects of bloat score on in vitro ruminal gas production and biofilm production when incubated with ground wheat forage substrate and mixed rumen microorganisms obtained from heifers grazing wheat forage during the vegetative stage of growth

Item[1]	Bloated	Nonbloated	SEM	*P*-value
Ruminal gas production				
Rate, c (cm^3/h)	6.0	3.8	0.54	0.01
Potential gas, a+b (cm^3/12 h)	15.0	14.7	0.308	0.56
Frothy foam production (cm^3/h)	0.31	0.26	0.02	0.01

[1]Least squares means (n = 4) for in vitro rumen fermentation with plant tannin treatment.

Biofilm production (g/mL) was similar ($P = 0.16$) among treatments. Rumen fluid obtained from elevated doses of quebracho condensed tannins (0, 1, and 2% tannins) incubated with minced fresh wheat forage in vitro decreased rate of gas and biofilm productions (Min et al., 2006b).

Frothy foam production (cm^3/h) and in vitro gas production from bloated and nonbloated heifers grazing winter wheat forage relative to bloat status are shown in Table 4 and Figure 1. During incubation period, the rate of gas and foam production was less ($P < 0.01$) from nonbloated than bloated animals (Table 4). Maximal foam generation in bloated and nonbloated animals (Figure 1) occurred during to the first 3 h of the in vitro incubation periods, and then gradually stabilized as wheat substrate was digested. Foam production was 50 to 70% greater in bloated than nonbloated rumen fluid during this time period. Lippke et al. (1969) reported the high correlation (r = 0.884) between in vitro gas production and foam stability during 3 h incubation with fresh alfalfa. Associated intraruminal pressures increased up to 70 mmHg or 9.34 kPa (Lippke et al., 1972), leading to eructation cessation. Collectively, these data suggest that frothy bloat onset more likely occurs within 1 to 3 h after consuming bloat-prone wheat forage.

Molecular PCR enumeration techniques were used to phenotypic analysis 5 major ruminal bacterial strains from rumen samples pooled from 4 head in the control, mimosa, and chestnut treatments (Figure 3). The relative abundance from the control rumen fluid of *F. succinogenes*, *S. bovis*, and *P. ruminicola* strains was similar on d 0, 10, and 25, whereas *R. flavefaciens* and *E. ruminantium* detection signals increased with time as a wheat forage advanced from vegetative to reproductive stage of development. In the presence of mimosa tannins in the diet, *S. bovis* and *P. ruminicola* populations were generally constant, but *F. succinogenes* and *R. flavefaciens* populations became dominant or recovered with time. *Eubacterium ruminantium* was not well detected over the grazing period. With supplementation with chestnut tannins, bacterial densities of *F. succinogenes* and *S. bovis* decreased with time, but the signal of *P. ruminicola* density intensified with time. Strains of both *R. flavefaciens* and *E. ruminantium* had relatively smaller densities and varied with time.

The 16S rDNA-PCR technique allowed visualization of 5 bacterial populations in the rumen of cattle associated with supplementation of mimosa and chestnut tannin supplementation. Earlier, Min et al. (2005a) reported that the relative proteolytic activities and specific bacterial growth and their responses to condensed tannins were influenced by dose level of tannins in vitro. In addition, the total number of cellulolytic bacteria, including populations of *F. succinogenes* and *Ruminococcus* spp., was significantly less in sheep supplemented with high tannin-containing calliandra forage (McSweeney et al., 2001). The present study shows *F. succinogenes*, *S. bovis*, and *P. ruminicola* strains were relatively stable over the time (d 0, 10, and 25) in the rumen of heifers grazing wheat forage without tannin supplementation. However, populations of *P. ruminicola* with chestnut tannin supplementation and both *F. succinogenes* and *R. flavefaciens* with mimosa supplementation increased. Conversely, populations of both *F. succinogenes* and *S. bovis* were decreased in chestnut tannin supplementation over time, indicating that these microbial populations in the rumen may also be dependent upon available nutrients in the tannin-containing diet as well as by tolerance to tannins per se (McSweeney et al., 2001).

Growth of ruminal bacteria (*Butyrivibrio fibrisolvens*, *Eubacterium* spp., *Ruminobacter amylophilus*, and *S. bovis*) was reduced by condensed tannins, but a strain of *P. ruminicola* was tolerant of condensed tannins (<400 to 600 μg/mL) from sainfoin (*Onobrychris viciifolia*; Jones et al., 1994) and birdsfoot trefoil (*Lotus corniculatus*; Min et al., 2005a). Furthermore, several species, including *S. gallolyticus*, *Clostridium* sp., and *Proteobacteria*, have been identified as tannin-tolerant bacteria (Brooker et al., 1994; Nelson et al., 1998). It appears from current studies that individual bacterial populations widely differ in their tolerance of tannins as well as source of tannins in cattle grazing wheat forage. The principal reasons for inhibitory effects of tannins are through inhibition of microbial enzymes, alteration of microbial metabolism, and deprivation of the substrates for microbial growth (Scalbert, 1991).

Across the grazing season, winter wheat forage mass on offer was gradually increased ($P < 0.01$) with time (Table 5). Forage DM, OM, and NDF were increased, whereas IVDMD was decreased with grazing season. Total CP ($P < 0.01$) and NPN ($P = 0.06$) also decreased with time, whereas insoluble protein-N increased

Table 5. Plant biomass, nutrient contents, and IVDMD of wheat forage from during the vegetative stage of growth (February 13 to March 19, 2007) in Vernon, TX

	Chemical composition (d)			
Item[1]	0	10	25	SEM
Forage biomass (kg of DM/ha)	468	584	848	71.51
Forage nutritive value				
Forage DM (%)	89.1	89.8	89.6	0.13
OM (g/kg of DM)	879	880	895	2.54
NDF (%)	33.6	48.8	40.1	0.57
IVDMD (%)	87.8	86.9	83.8	0.12
Protein fraction (% of DM)				
Total CP	27.7	22.5	21.2	0.77
Soluble protein-N	54.1	51.8	53.0	2.44
Insoluble protein-N	38.8	42.4	40.9	2.31
Soluble nonprotein-N	7.1	5.8	6.0	0.31

($P = 0.07$) during the grazing period. Plant-soluble protein was relatively similar ($P = 0.19$) through the grazing period.

Daily supplementing mimosa and chestnut tannins improved ADG of beef heifers and decreased bloat frequency with minimal impact to the microbial populations studied. This study has shown that wheat pasture bloat is related to increased in vitro production of frothy foam and ruminal gas. Experiment 2 demonstrated that some ruminal bacterial species are variably tolerant to intolerant of differing sources of tannins. Chestnut tannin supplementation effectively decreased frothy bloat and increased ADG in stocker cattle-wheat systems.

IMPLICATIONS

The present work showed positive effects of plant tannins on controlling frothy bloat and modifying bacterial populations in the rumen and consequently improved animal performance. Different types of tannins have different modes of action on rumen bacterial populations. This suggests that plant tannin supplementation is effective for extenuating frothy bloat and improving animal performance without negative effects on heifers grazing winter wheat forage.

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